

## ITALIAN CHEESE RIPENING. V. VARIOUS FREE AMINO AND FATTY ACIDS IN COMMERCIAL ROMANO CHEESE<sup>1,2</sup>

The gross chemical changes that occur during the ripening of Romano cheese have been reported in previous papers of this series (2, 3). Although these investigations indicate that both fat and protein degradation is important in the ripening of this cheese, no information was presented as to the specific chemical compounds that are related to characteristic flavor development. In another paper (4) data were presented for various free amino acids in Provolone cheese; they showed that both free butyric and free glutamic acid are associated with the development of the characteristic flavor of this cheese. A related study was conducted on Romano cheese and the findings are presented in this paper.

### PROCEDURE

Fourteen samples of commercial Romano cheese of different ages were obtained from various domestic factories making Italian cheese. Seven of the cheeses were 4 months old, three were 10 months old, three were 18 months old, and one was 26 months of age.

The manufacturing of the seven 4-month-old cheeses was observed by visiting each of the five factories which made these cheeses. The methods used by the different plants varied slightly but all were considered as being representative commercial procedures. After salting, the cheeses were cured at The Ohio State University under the same conditions of temperature (50° F.) and humidity (70% relative humidity).

The other seven cheeses were obtained directly from the manufacturers after they had been cured under the commercial conditions in the factories where they had been made. A history of each cheese was obtained.

The cheeses were sampled by removing a wedge about 2 in. wide at the outer edge. The outer 1/4 in. of rind was discarded, and the cheese was ground in a rotary hand grater, packed in air-tight containers, and stored at -15° C. until analyzed. Analyses for moisture and water-soluble nitrogen were made within 48 hours in the same manner as previously reported (4).

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<sup>2</sup> Journal Article 39-55, Ohio Agricultural Experiment Station, Wooster.

TABLE 1  
General characteristics of Romano cheese

Sample No.	Age (mo.)	Flavor		Source and type of enzyme product <sup>b</sup>	Moisture (%)	Water soluble nitrogen (% of total N)
		Intensity (C.F.) <sup>a</sup>	Comment			
1	4	0.5	Mild	Purified calf and kid rennet paste	33.1	15.4
2	4	1	Acidic	Crude mixed calf and kid rennet paste	35.2	13.4
3	4	2	Clean	Kid glandular preparation	34.4	23.4
4	4	2	Sl. unclear	Kid rennet paste	34.7	17.2
5	4	2	Sl. bitter	Calf rennet paste	33.8	14.8
6	4	3	Sharp, goaty	Kid glandular preparation	31.8	12.9
7	4	3	Piquant, sharp	Lamb glandular preparation	35.6	20.9
8	10	1	Mild, acidic	Purified calf rennet paste	32.8	20.4
9	10	1	Medicinal, mild	Calf and kid glandular preparation	33.8	24.4
10	10	2½	Clean	Kid glandular preparation	34.0	21.0
11	18	1	Mild	Rennet extract	33.6	21.3
12	18	2	Sl. sweet	Calf glandular preparation	34.0	18.0
13	18	3	Sharp, goaty	Kid glandular preparation	33.6	—
14	26	4	Ideal	Kid rennet paste	32.9	15.4

<sup>a</sup> C.F. = characteristic flavor; 0 = no flavor; 4 = ideal, highest characteristic flavor.

<sup>b</sup> Rennet extract was used with all glandular preparations.

A portion of the water extract, which had been prepared according to the method of Sommer and Harper (7), was concentrated ten to one under vacuum (20 mm.) at low temperature (5° C.). This concentrate was used within 24 hours for amino acid analyses. Analyses for representative free amino acids (aspartic acid, glutamic acid, glycine, alanine, threonine, histidine, valine, methionine, and the combined leucines) were made by the paper chromatographic procedure of McFarren (6) as described earlier (4, 5).

Analyses were made also for acetic, propionic, and butyric acids with 5 g. of cheese by a direct silica gel chromatographic procedure (1).

## RESULTS

The ages, characteristic flavor intensities, sources and types of enzyme utilized in manufacture, moisture, and water-soluble nitrogen content are given in Table 1. The samples are arranged in order of increasing age, and those of the same age are arranged in order of increasing flavor intensity. As reported in a previous paper (3), the flavor intensity was generally related to the type of enzyme products used in the manufacture of the cheese. Also, the results confirmed those previously reported for Provolone cheese in that there was no definite relationship between the water-soluble nitrogen and the characteristic flavor intensity of the Romano cheese (3). The highest water-soluble content was found in Sample 9, which had one of the lowest flavor intensities, whereas the lowest water-soluble nitrogen value was found in Sample 6, which had a relatively high degree of characteristic flavor. The moisture contents of the cheeses were in general agreement with those previously reported.

The results of the analyses for the various free amino acids are presented in Table 2. The samples are arranged in the same order as in Table 1. Qualitatively, all samples contained aspartic acid, alanine, glycine, glutamic acid, leucines, and valine, whereas some samples did not have any detectable quantities of histidine, methionine, and threonine.

The quantitative values reveal wide variations in the concentration of a given acid in cheeses of the same or different ages. The seven 4-month-old cheeses generally had a lower concentration of aspartic acid, glycine, and methionine, whereas the seven samples 10 months old or older contained less histidine and threonine. On the other hand, the concentrations of alanine, glutamic acid, leucines, and valine did not appear to be related to variations in age of the cheese. With the exception of glutamic acid, none of the amino acids revealed any definite relationship to characteristic flavor development. Glutamic acid was generally high in samples receiving a relatively high flavor intensity score. For example, the cheeses with a CF-2 or higher had a glutamic acid range of 4.8 to 6.3 mg. per gram solids with an average value of 5.5; whereas those with a CF less than 2 had a glutamic acid range from 2.1 to 6.0 mg. per gram solids and an average of 3.4 mg. per gram solids.

The amounts of various free fatty acids in the 14 commercial Romano cheeses are given in Table 3. Acetic, propionic, butyric, and the group designated as "higher" acids were present in all samples, although the concentration varied

TABLE 2  
The free amino acid content of commercial Romano cheese

Sample No.	Age (mo.)	Flavor intensity (C.F.) <sup>a</sup>	Aspartic acid	Alanine	Glycine	Glutamic acid	Histidine	Leucines	Methionine	Threonine	Valine
						(mg. per gram cheese solids)					
1	4	0.5	+	1.2	+	3.8	7.0	8.2	+	1.5	3.0
2	4	1	0.6	1.1	+	2.1	7.6	8.3	0	0	2.6
3	4	2	0.9	1.6	+	4.8	6.0	9.7	+	1.2	3.9
4	4	2	+	2.1	0.7	5.3	4.2	9.1	0.3	0.8	3.1
5	4	2	1.1	0.8	+	5.0	2.8	9.6	+	0	3.5
6	4	3	+	1.6	+	—	4.1	7.5	0.2	1.8	3.7
7	4	3	1.7	1.3	0.8	5.7	3.6	8.9	+	0.9	3.6
8	10	1	2.0	—	1.3	3.0	8.0	9.7	0	1.8	4.2
9	10	1	1.4	1.7	0.5	2.4	6.0	9.6	0	2.0	5.5
10	10	2.5	1.3	1.1	1.2	6.3	0	12.5	+	0	3.4
11	18	1	2.9	1.2	+	6.0	0	9.9	0.73	0	3.9
12	18	2	1.6	3.5	0.9	5.7	4.9	9.0	0.7	0	4.0
13	18	3	2.6	4.0	0.8	5.4	2.9	10.7	0.7	0	4.3
14	26	4	0.9	1.2	+	5.6	0	8.3	0.6	0	3.6

<sup>a</sup> C.F. = characteristic flavor; 0 = no flavor; 4 = ideal, highest characteristic flavor.

TABLE 3  
*The free fatty acid content of commercial Romano cheese*

Sample No.	Age	C.F. <sup>a</sup>	Acetic	Propionic	Butyric	Higher <sup>b</sup>
	(mo.)		(mg. per gram cheese solids)			
1	4	0.5	0.2	0.05	0.8	2.7
2	4	1	0.86	0.21	1.7	6.4
3	4	2	0.20	0.16	1.5	5.0
4	4	2	0.27	0.18	1.8	6.1
5	4	2	0.31	0.23	1.1	4.7
6	4	3	0.26	0.14	3.0	8.1
7	4	3	0.29	0.22	3.2	6.3
8	10	1	0.56	0.30	0.9	2.3
9	10	1	0.32	0.26	2.1	8.4
10	10	2.5	0.24	0.18	3.6	10.1
11	18	1	0.15	0.11	0.8	3.1
12	18	2	0.41	0.34	2.8	9.3
13	18	3	0.30	0.24	6.4	10.9
14	26	4	0.41	0.26	7.1	14.7

<sup>a</sup> See footnote on Table 1.

<sup>b</sup> Includes all fatty acids above 5 carbon atoms; a molecular weight of 150 was assumed to calculate higher acid concentration.

widely among the different samples. The amounts of acetic and propionic acids could not be related to the age of the samples and were present in lower concentrations than butyric acid. Generally, the butyric acid content of the 4-month samples was less than that of the samples 10 or more months of age. Similarly, the higher fatty acids were usually higher in the older cheeses. The exceptions to the age relationships, Samples 8 and 11, can be explained on the basis of the enzyme system used in manufacture. Sample 11 was made with rennet extract only, and Sample 8 was made with a purified rennet paste. These samples contained 0.8 and 0.9 mg. butyric acid per gram cheese solids, respectively. Sample 1, which was the only other sample made with a purified rennet paste, was also the only sample with less than 1 mg. of butyric acid per gram of cheese solids. This relationship of enzyme product to the butyric acid and higher acid values was in accordance with findings in earlier studies involving the volatile acids formed in Romano cheese (3).

Generally, the butyric acid content of the Romano cheese was related to the characteristic flavor intensity. However, there were more exceptions to this relationship than previously shown between butyric acid and Provolone cheese flavor (4). Cheeses with a CF-1 contained from 0.8 to 2.1 mg. of butyric acid per gram cheese solids, whereas cheese with a CF-2 contained from 1.1 to 2.8 mg. of butyric acid. The cheeses with a CF value above 2 contained from 3.0 to 7.1 mg. of butyric acid. The higher fatty acids also were found to be related generally to the flavor intensity with such exceptions as Sample 2 with a CF-1 and 6.4 mg. of higher acids, as compared to Sample 7 with a CF-3 and 6.3 mg. of higher acids. Sample 9 with a CF-1 contained 8.4 mg. of higher acids as compared to Sample 6 with a CF-3 and 8.1 mg. of higher acids.

In the previous paper of this series dealing with Provolone cheese, a definite relationship was found between glutamic acid and butyric acid content as related

TABLE 4  
*The relationship of characteristic flavor to the free butyric and  
glutamic acid content of commercial Romano cheese*

C.F. <sup>a</sup> 0.5 and 1.0		C.F. 2.0 and 2.5		C.F. 3.0 and 4.0	
Butyric	Glutamic	Butyric	Glutamic	Butyric	Glutamic
(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)
0.8	3.8	1.1	5.0	3.0	5.7
0.9	3.0	1.5	4.8	3.2	5.4
0.8	6.0	1.8	5.3	6.4	5.6
1.7	2.1	2.8	5.7	7.1	5.6
2.1	6.3	3.6	2.4		

<sup>a</sup> C.F. = characteristic flavor. Recorded from 0 to 4.

to flavor intensity (4). In order to determine if such a relationship might exist also in Romano cheese, the butyric acid and glutamic acid values are compared to flavor intensity in Table 4. Some of the exceptions to the relationship of either glutamic or butyric acid to flavor intensity can be explained by the data in this table. For example, in the first column the cheese with a glutamic acid value of 6.0 had a corresponding butyric acid value of 0.8. In the same column, the fourth sample had a relatively high butyric acid value (1.7) but a relatively low glutamic acid value. However, there is no clearly defined relationship between glutamic acid and butyric acid and flavor development, as was noted for Provolone cheese.

#### DISCUSSION

The results of this investigation on Romano cheese are in general agreement with those for Provolone cheese, which were reported previously (4). These two cheeses are closely related in that they depend upon the same bacterial starters and on the same lipolytic enzymes and differ primarily in the heat treatments which they receive during manufacture. The free amino acids and free fatty acid contents of Romano cheese were generally higher than those of Provolone cheese, which is probably explained by the high heat treatment which Provolone cheese received during molding.

In the present study the variability in the concentrations of the free fatty acids could be related to the type of lipase system used in the manufacture of the cheese, confirming the earlier results on Provolone (4). In the present study, insufficient data were available concerning the starter cultures used in the cheese to permit determining whether or not any relationship existed between the type of starter organisms and the accumulation of the various free amino acids. However, the microbial flora is undoubtedly important in determining which amino acids accumulate during the cheese ripening, and further studies are being directed to the bacterial enzymatic mechanisms involved in the ripening of Romano cheese.

Much time and effort have been expended by many research workers to determine the chemical compounds related to flavor development of cheese. Ideally, analyses should be made simultaneously for as many different types of com-

pounds as possible, but the time limitations have made this impossible. Therefore, analyses generally have been limited to one group of compounds. The approach used in this study has been to study representative compounds of different groups. The interrelationship observed between an amino acid (glutamic) and a fatty acid (butyric) suggests that analyses including compounds of other groups, such as carbonyls, alcohols, and amines, could provide further information concerning the cheese ripening process.

The fact that a relationship between flavor and the butyric acid-glutamic acid content exists does not prove that such compounds are necessarily flavor compounds. They may be precursors of flavor constituents or merely associated with other flavor compounds. In Provolone cheese, there was a definite relationship between the flavor intensity of 30 samples of cheese and the combined butyric-glutamic acid content. In the present study, there is an indication of this relationship, but the irregularity of the results does not permit a definite conclusion in this respect. The most probable explanation is that a third compound or compounds may be interrelated with the butyric and glutamic acids. This hypothesis is strengthened by the fact that the group of higher acids appears to be related to the flavor of this cheese.

Both butyric and glutamic acid could be subject to further degradation to form other compounds, which may explain the role of the former compounds in cheese flavor. On the other hand, it is possible that amino acids and fatty acids might form a salt complex which would have flavor imparting characteristics. The butyric-glutamic relationship in Provolone and the propionic-proline relationship in Swiss cheese suggest this possibility (4, 5).

#### SUMMARY

Various free amino and fatty acids were measured in 14 samples of commercial Romano cheese. The amounts of some of the free amino acids could be related to the age of the cheese, but only the free glutamic acid could be related to the flavor intensity of the cheese. Acetic and propionic acids were not associated with either the age of the cheese or flavor intensity, whereas both the higher acids and butyric acid were generally related to age and the flavor intensity of cheese of the same age.

In general, the free butyric acid and higher fatty acid contents were dependent upon the type of enzyme product used in the manufacture of the cheese, whereas the free amino acid concentrations were not associated with the lipase product added to the milk.

The results of this study on Romano cheese are in general agreement with those presented earlier for Provolone. The major difference noted was that the amounts of free acids for cheese of the same relative flavor intensity are much higher in the Romano cheese. There were wide variations in the amounts of a given free amino or fatty acid in cheese of the same age. This may be attributed to the differences in manufacturing practices in the various factories making Romano cheese.

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